Sergio Grinstein: Phagocytosis step by step

Grinstein studies how phagocytes clean up pathogens and the mess left behind by cell death.

Macrophages are cells of the immune system that are specialized to carry out phagocytosis—the uptake of large particles by cells. Like a combination security guard and janitor, macrophages patrol the body, voraciously consuming both microbes and the corpses of apoptosed cells to keep tissues functioning smoothly.

Sergio Grinstein has spent much of his career working to provide a step-by-step explanation of phagocytic processes. This effort has entailed expanding on his early training in ion transport (1) to understand the electrophysiology of the phagosome (2, 3). It's also led him to study seemingly wide-ranging topics such as the behavior of membrane lipids (4) and individual cell surface receptors (5). We called him at his lab at Toronto's Hospital for Sick Children to talk about the major steps in phagocytosis and in his career.

INITIAL ENCOUNTER

You grew up in Mexico…

I was born and grew up in Mexico City and did all my schooling there, all the way through to my PhD. It was a very pleasant place to live, and the weather was marvelous—you could see the volcanoes. Of course, at that time, Mexico City was much smaller and cleaner than it is now.

> "All of a sudden we're learning things that we never expected to find."

Both of my parents were first-generation immigrants from Poland who came to Mexico fleeing anti-Semitism in Europe. In the 1920s and '30s, both Canada and the US had pretty much closed their borders to Jewish immigrants, so my parents and many other Jewish emigrants ended up in Latin America.

When my father arrived in Mexico, he didn't have a profession, so he picked up selling shoes. But he always wanted his kids to be professionals and academics, if we possibly could. That's why one of my brothers is a physicist, another is an actuarial mathematician, and I'm a biologist.

How did you come to Toronto for your postdoc?

I had met my wife while I was an undergraduate and she was still in high school. We grew up together and both wanted a career in science, so our priority obviously was to find one city where we could find two postdocs. We applied to a bunch of places, and we were really fortunate that we had three different offers in three different places, one of which was Toronto.

At first, we planned to complete our postdocs and then return to Mexico after a couple of years. We had even lined up some job offers in Mexico. But toward the end of our second year we decided to forgo those offers and look for something else, even though finding two positions together was very hard. We finally took positions together in Switzerland, but, shortly after we got there, I was offered a position as an independent researcher here at SickKids in Toronto, and then my wife was also offered a job as a research associate in Toronto. So we spent a year in Switzerland and then returned to Toronto. A year later my wife got her own position as an independent researcher, and we've happily stayed here ever since.

TAKING IT UP

When did you first conceive an interest in ion transport?

My PhD studies were on the effect of insulin on ion transport in muscle and epithelial cells, and in my postdoc I worked on something similar—ion transport in red blood cells. At that time, to be honest, I didn't have any enlightened vision about ion transport. It was just what was being studied in the labs I'd joined.

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Sergio Grinstein

When I first started as an independent researcher, I thought it would be a good idea to work in a different area than my postdoctoral supervisor, so I proposed something totally different from what I had been doing. It was totally crazy, but I got funding for it! [Laughs] In retrospect, though, it wasn't a very smart thing to do; the first six months were terribly frustrating and depressing. I was actually ready to give up science and go back to Mexico and do something else entirely. But mercifully, I survived that stage because a colleague two floors up, an immunologist, came to me with a problem that he wanted to discuss about effects of ions on lymphocytes. At the time, immunologists didn't do very much physiology or cell biology. I realized that with my expertise in physiology, pH, and ion transport there was a unique niche for me within the immunological community.

Part of that niche involves studying pH regulation in phagosomes…

When a macrophage or neutrophil encounters a bacterium, yeast, or fungus, the immune cell takes that pathogen into a specialized cellular structure called a phagosome, where it is inactivated, killed,

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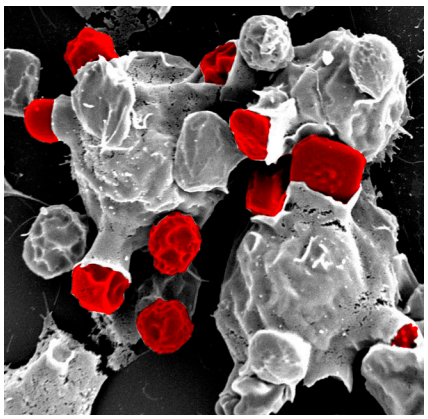

IMAGE COURTESY OF ROBERT TEMPKIN

Macrophages (white) engulf red blood cells (red) in this scanning EM image.

and degraded. But when a phagosome is first formed, the conditions inside it are pretty innocuous because it's just a vacuole composed of the plasma membrane, enclosing a little bubble of the extracellular space. In order to kill what's inside, you have to convert both the membrane of that vacuole, and especially the vacuole's interior, into a really hostile environment that is microbicidal. That happens through a process called phagosomal maturation, which involves a very complex series of fusion reactions that bring in membranes from the endocytic pathway, countered by fission events that remove certain components from the vacuole.

One thing that arrives with endocytic membranes is the vacuolar ATPase, which pumps protons into the compartment to make it more acidic. Within about half an hour, V-ATPases take the vacuole interior from a neutral or maybe slightly alkaline pH down to a pH of five or less. A couple of years ago, we showed that phagosomes' electrical neutrality is maintained in part by moving one positive cation out of the phagosome for each proton that's pumped in. The extreme acidity of the phagosome works together with phagosome proteins that help kill and degrade the target particle. Of course, some pathogens are capable of escaping being killed, and they each use different mechanisms to do so. That is also something we've been working to understand.

BREAKING IT DOWN

How do you choose what research subjects to focus on?

You have to be opportunistic and try to apply general principles to different biological problems. For example, macrophages are now known to be important in many pathological processes including heart disease, Alzheimer's, diabetes, infectious diseases, and obesity. We can't be experts in all these fields, but we can use our expertise to study some aspects of them.

> "I think it's overly simplistic to assume that all instances of phagocytosis are the same."

In the last couple of years, we've started studying one of the major lipids in the plasma membrane, phosphatidylserine. Relatively little is known about this lipid because there haven't been any easy ways to study it. We devised a probe to measure phosphatidylserine in intact cells, and all of a sudden we're learning things that we never expected to find.

Another thing that we started doing in the last few years is studying single receptors on the surface of immune cells and trying to measure how they behave. For years, everybody took it for granted that proteins could freely float within the lateral plane of the plasma membrane like corks on the sea. But today it's known that there are corrals and barriers in the membrane, which has interesting implications for receptors—such as those involved in phagocytosis—that become activated by lateral clustering in the plane of the membrane.

Finally, we're becoming interested in the different kinds of phagocytosis carried out by macrophages. Some phagocytic processes, such as taking up bacteria, result in inflammation, but others, including cleaning up the debris left by apoptosed cells, do not. I think it's overly simplistic to assume that all instances of phagocytosis are the same.

Do you have any advice for young scientists?

I think the era of the specialist, the time of looking at a single molecule or gene, is long past. Now, the lumpers, the integrators, the people who can synthesize multiple concepts, are taking precedence, so it's important to keep your mind open to more complex and elaborate systems. Also, you can't follow a technique anymore. You have to follow a biological question instead, and, if you don't have a technique you need, learn it. The more you have under your belt in terms of technical expertise, general and basic knowledge, the better you're going to do.

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Grinstein and his wife have enjoyed lifelong travels together, including a trip to Jerusalem.